

Reversed Phase-High Performance Liquid Chromatographic Method to Measure Migration of Semivolatile Compound, Vanillin, in Ipratropium Bromide Inhalation Solution

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ABSTRACT Ipratropium bromide, a bronchodilator, is used as an inhalation solution. Commercial ipratropium bromide solution products are packaged in low-density polyethylene (LDPE) vials, through which semivolatile compounds are reported to migrate. In this article, a specific reversed phase-high performance liquid chromatographic method to assay vanillin, a semivolatile compound, in ipratropium bromide solution is described. The method was validated for a concentration range for vanillin from 30 ng/mL to 1,600 ng/mL.

Migration of vanillin was assessed in two commercial preparations, ATROVENT® (ipratropium bromide) Inhalation Solution packaged in a secondary foil pouch and a generic ipratropium bromide inhalation solution packaged in a carton. Levels of vanillin detected in ATROVENT® after 6 months of storage at 40° C and 75% RH were below the limit of detection (11 ng/mL). Significant migration of vanillin was observed after 1 month in the generic product and reached 165 ng/mL to 999 ng/mL in three months under the same storage conditions.

It is concluded that this method can be readily used to measure vanillin in commercial preparations of ipratropium bromide inhalation solution. The results strongly indicate that a protective secondary packaging material is critical in preventing migration of semivolatile compounds. This study result is in agreement with the FDA's recommendation to consider even the secondary packaging components as potential sources of contamination and the use of an overwrap (typically aluminum foil) to decrease the overall permeability.

INTRODUCTION

Ipratropium bromide is a beta-agonist used alone to treat chronic obstructive pulmonary disease or in combination with other bronchodilators to treat pulmonary conditions, including asthma, chronic bronchitis, and emphysema (1,2). The safety and therapeutic benefits derived from ipratropium bromide and similar drug products depend on the quantity and properties of the active drug substance itself, which may be compromised by contaminants and impurities it contains. Ipratropium bromide inhalation solutions are commonly packaged in primary container systems composed of vials made from a low-density polyethylene (LDPE) resin (2,3). The LDPE vials themselves are known to have low permeation resistance and allow volatile and semivolatile contaminants to migrate into solutions (4). Routine analytical profiling of a drug product is cited as an important part of the International Harmonization Guidelines (5) and in other authoritative sources (6) because of the potential adverse effect of impurities and/or contaminants in drug products.

The Food and Drug Administration (FDA) has prepared a guidance document recommending that appropriate secondary barrier packaging, such as aluminum foil, be used to reduce migration of volatile and semivolatile compounds into inhalation solutions packaged in LDPE vials (7). FDA regulations generally require that qualified analytical methods be used to support packaging system suitability and, where appropriate, accelerated stability studies be conducted to demonstrate packaged product integrity throughout the shelf life (8). Particularly, it is of interest to develop analytical methods that can be used to study permeation kinetics of semivolatile and volatile compounds through the LDPE packaging components into an inhalation solution.

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In the first part of this study, a reversed phase high performance liquid chromatography method was developed and validated to measure the semivolatile compound, vanillin, in a 0.02% ipratropium bromide inhalation solution. Vanillin was selected as a model compound because it is present in secondary packaging systems (such as paper cartons and shippers) commonly used in commercial inhalation solution products (3,9). In the second part of the study, this method was applied to measure vanillin levels, due to migration, in ipratropium bromide products (2,3).

MATERIALS AND METHODS

The chromatographic method was specifically developed to quantify vanillin in the presence of ipratropium bromide and tropic acid, the active and known degradation product, respectively. In Figure 1, structural and molecular formulas of the three ingredients are presented.

Chromatography was carried out on a phenyl column with the mobile phase consisting of 0.01 M dibutylammonium phosphate buffer, pH 2.5: methanol:tetrahydrofuran (92.8:4.9:2.3). HPLC details are presented in Table 1.

Method Development and Validation

The chromatographic method was validated by assessing linearity, accuracy, selectivity, limit of quantitation, and precision for vanillin. System suitability was acceptable based on (i) a resolution between ipratropium bromide and tropic acid of not less than 4.0 and (ii) a 20 ng/mL solution of vanillin that produced a response greater than 5 times baseline noise. During method development, separation of peak responses for vanillin without interference from placebo excipients was evaluated. Samples of ipratropium bromide solution with and without vanillin and placebo were injected on the HPLC system. The resulting chromatograms showed separation for all components, and the method displayed no significant bias to the determination of vanillin by the matrix. This method was used to measure vanillin in ipratropium bromide solutions.

Ipratropium Bromide Inhalation Solutions and Storage Conditions Used in the Study

Table 2 provides the summary of the qualitative composition, primary packaging, and secondary packaging system used in the two commercially

available ipratropium bromide inhalation products (2,3).

Ipratropium bromide inhalation solutions were placed as received in the shippers at 40° C and 75% relative humidity. ATROVENT® was evaluated initially, and after 1, 2, 3, and 6 months of storage using a composite sample at each time point. Five individual vials of the generic ipratropium bromide inhalation solution were evaluated initially and after 1, 2, and 3 months of storage.

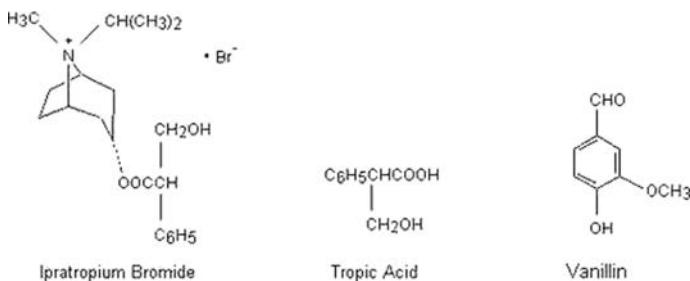


Figure 1. Structure formulas of ipratropium bromide, tropic acid, and vanillin

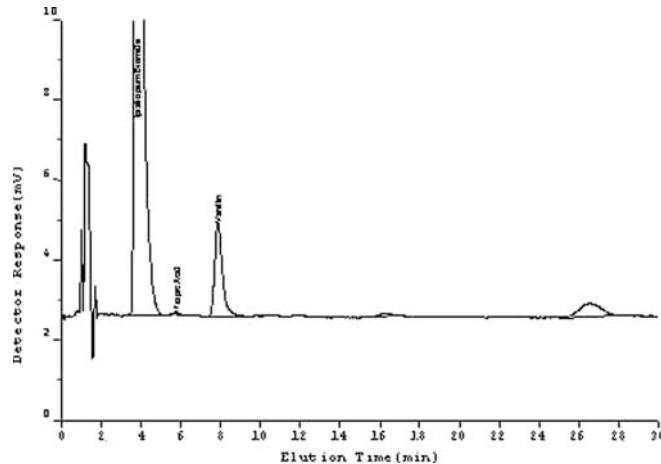


Figure 2. RP-HPLC chromatogram of ipratropium bromide, tropic acid, and vanillin

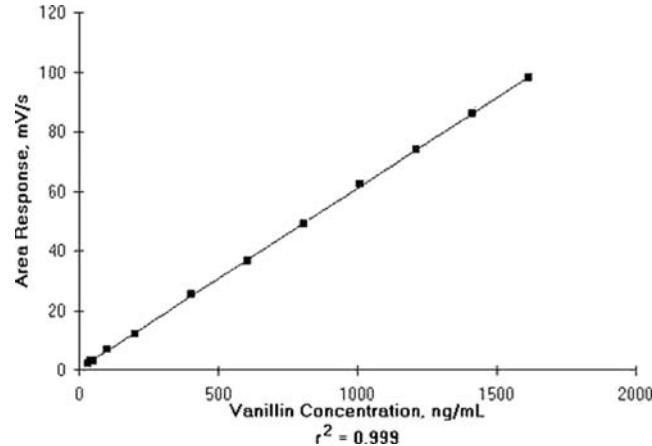


Figure 3. Linearity of vanillin in ipratropium bromide solution

Table 1. HPLC operating conditions

Mobile Phase	0.01 DBAP/Methanol/ THF (92.8/4.9/2.3)
Flow Rate	3.0 mL/min
Column Temperature	35° C
Injection Volume	50 µL
Run Time	30 min.
Detection	UV at 220 nm
Relative Retention Times	Ipratropium Bromide 1.0 Tropic Acid ~1.5 and Vanillin ~2.0

Table 2. Summary of composition and packaging configurations

Name of the Product	ATROVENT® (ipratropium bromide) Inhalation Solution	Ipratropium Bromide Inhalation Solution
Manufacturer	Roxane Laboratories, Inc.	Dey Laboratories
Ipratropium Bromide	0.02%	0.02%
Ingredients	Sterile, preservative free, isotonic solution, pH adjusted 3.4 (3 to 4) with hydrochloric acid	Sterile, preservative free, isotonic solution, pH adjusted 3.4 (3 to 4) with hydrochloric acid
Volume	2.5 mL	2.5 mL
Primary Packaging	Each vial is made from LDPE resin. Debossed	Each vial is made from LDPE resin. Paper label adhered to vial.
Secondary Packaging	Foil pouch /Carton / Shipper	Carton / Shipper

Table 3. Vanillin concentration (ng/mL) in the commercial ipratropium bromide solution products

Product	Lot	Vanillin, ng/mL				
		Initial	1 Month	2 Month	3 Month	6 Month
ATROVENT® (ipratropium bromide) Solution						
962101	None Detected	None Detected	None Detected	None Detected	None Detected	None Detected
962102	None Detected	None Detected	None Detected	None Detected	None Detected	None Detected
Generic Ipratropium Bromide Solution						
Lot N 033	None Detected	53 to 155	96 to 537	328 to 999		
Lot N 015	None Detected	<30 to 48	153 to 215	191 to 213		
Lot N 025	None Detected	41 to 213	122 to 576	185 to 811		
Lot N 021	None Detected	<30 to 209	127 to 708	306 to 833		
Lot B 736	None Detected	<30 to 121	47 to 448	165 to 548		

RESULTS

A representative chromatogram of a solution containing ipratropium bromide 0.02%, tropic acid 0.0006% and vanillin, 999 ng/mL is shown in Figure 2. The chromatogram shows that the peaks are well characterized and meet acceptability criteria for peak separation.

In Figure 3, concentration of vanillin, ng/mL versus the peak response is plotted. The study results shown in Figure 3 demonstrate that the peak detection response of vanillin was directly proportional to concentration over the range of 30 to 1600 ng/mL. The percentage recovery of vanillin from solutions containing hydrochloric acid, sodium chloride, and ipratropium bromide was from 94.5 to 111.0 with an average of 103.4% and RSD of 5.1%. RSD results for vanillin demonstrated a precision of 11.7, 2.1, and 1.3% for concentrations of 30, 800, and 1,600 ng/mL, respectively. An LOQ of 30 ng/mL was determined by the lowest concentration that gave acceptable accuracy and precision. Based on these findings, the above-described method is validated for

vanillin over the range of 30 to 1,600 ng/mL in ipratropium solution.

Vanillin in the Commercial Products

Table 3 shows concentrations of vanillin in ng/mL determined over the study period for several lots of two commercial ipratropium bromide solution products.

The vanillin levels in the ATROVENT® samples were below the limit of detection (11 ng/mL) throughout the 6-month study period. Vanillin was measurable in the generic product after storage for one month and continued to increase throughout the study period. It was also observed that there was considerable variation in the measured amounts of vanillin in the five different vials of the generic product.

DISCUSSION

Analytical development and validation studies demonstrate that this method for assay of vanillin in ipratropium bromide inhalation solutions of 0.02% is linear, selective, and quantitative. The method is free from interference from placebo excipients and the known degradation product of the ipratropium bromide formulations. The linear concentration range for vanillin was established from 30 ng/mL to 1,600 ng/mL, with an average recovery rate of 103.4%.

This method was used to evaluate the presence of vanillin in ipratropium bromide solution packaged in LDPE vials. It was observed that there is a major difference in the secondary packaging. Vanillin was not detected in samples of drug product packaged with a foil pouch. Increasing amounts of vanillin, 165 ng/mL to 999 ng/mL, were measured in the drug product packaged without a protective barrier (see Table 2).

It is theorized that many factors may influence the permeation of vanillin, including uniformity of vial wall thickness, amount of vanillin in shippers, position of the vials in the cartons, the paper labels adhered to vials, and migration kinetics of vanillin from shippers to the solution. The major differences in the packages tested in this study were (i) a protective foil barrier and (ii) the use of paper labels. Additional studies in this laboratory have shown that vanillin does not migrate through a foil barrier. As

pointed out earlier, a major source of vanillin is related to cardboard used in cartons and shippers. Therefore, this research strongly suggests that the vanillin present in the shipper and carton migrates through the LDPE vials into solution. Although this research did not attempt to study the contribution of each secondary packaging component and kinetics of migration of vanillin through LDPE into ipratropium solutions, the researcher can apply this analytical method to develop a kinetic model. These models are extensively described in the pharmaceutical literature (10) and are used for development of secondary packaging systems.

This study has revealed that vanillin readily migrates through LDPE resin, a primary packaging component used in many inhalation products. This research concludes that the composition of secondary packaging material is critical in preventing the migration of semivolatile compounds. The study results support the FDA's position that secondary packaging components are an important part of a suitable packaging system for inhalation solutions and recommend that an overwrap (typically aluminum foil) be used to decrease overall permeability (11).

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